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Research Article

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Hepatoprotective and antioxidant activity of ethanolic extract of *Anisochilus carnosus* whole plant against paracetamol induced liver injury

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ABSTRACT

The ethanolic extract of the whole plant of Anisochilus carnosus (Lamiaceae) was evaluated for hepatoprotective activity in wistar rats with liver damage induced by paracetamol. The extract of an oral dose of (200 mg/kg, bw and 400 mg/kg, bw p.o.) exhibited a significant protective effect against paracetamol induced hepatotoxicity by lowering the serum levels of aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), total serum bilirubin, and malondialdehyde equivalent, an index of lipid peroxidation of the liver. The activity of the ethanolic extract of Anisochilus carnosus whole plant was comparable to the standard drug, silymarin (100 mg/kg, p.o.), and the histopathological studies supports the above finding. Results indicate that Anisochilus carnosus possesses significant hepatoprotective effect on paracetmol -induced hepatotoxicity in mice.

Keywords: *Anisochilus carnosus*, Antioxidant, Hepatoprotective; Paracetamol.

INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Liver, the key organ of metabolism and excretion has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Hence, this organ is subjected to a variety of diseases and disorders. Several hundred plants have been examined for use in a wide variety of liver disorders. Liver is the largest organ in the vertebrate body and the site for intense metabolism. Liver diseases remain one of the serious health problems and the Indian traditional system of medicine, especially Ayurveda have put forward a number of medicinal plants and their formulations for liver disorders. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost.

Anisochilus carnosus, (Lamiaceae) is an annual shrub found in various high altitude regions of India. *Anisochilus carnosus* is claimed to be useful in liver and stomach disorders, previously it was reported that the extracts of *Anisochilus carnosus* possess significant hepatoprotective activity against paracetamol-induced hepatotoxicity in rats. Volatile oil of *Anisochilus Carnosus* contains Carvacrol (27.9%), Camphor (14.1%) and α-cis bergamotene (10.2%). The plant has been traditionally used as hepatoprotective agent, stimulant, and anti-ulcer, anti-inflammatory [1][2] and[3]. The most important of these chemically active constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous, medicinal plants are also used for medicinal purposes [4][5]. Phytochemical investigations resulted in the isolation of ursolic acid, corosolic acid, 24-hydroxy

corosolic acid, maslinic acid and 3b, 7b, 24-trihydroxy-urs-12-en- 28-oic acid. In this study,we investigated the phytochemical content followed by the n-hexane extract of its leaves were subjected to chromatographic separation to afford two steroids, including stigmasterol and β-sitosterol. Both compounds were isolated from this species [6].

EXPERIMENTAL SECTION

Plant material and preparation of extract

The whole plant of the *Anisochilus carnosus, (Lamiaceae)* was collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of October 2015. The plant materials were authenticated in department of chemotaxonomiy at National Botanical Research Institute, Lucknow and voucher specimens were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference. The shade dried parts of whole plant of about 900 g were subjected for size reduction to coarse powder. The powder was then extracted with 90 % ethanol using Soxhlet apparatus. The ACWPE was concentrated under vaccum to get the residues and stored at – 20 ^{0}C in deep freezer till use.

Animals

Male Wistar rats (150-250 gm.) were procured from National Botanical Research Institute (Lucknow). They were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D [7].

Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [8]. Reaction mixture contained 60 µl of 1.0 mM $FeCl_3$, 90 µl of 1 mM 1, 10-phenanthroline, 2.4ml of 0.2 M phosphate buffer (pH7.8), 150 µl of 0.17M H_2O_2 and 2.5 ml of *Anisochilus carnosus* whole plant extract at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation: % Inhibition = $((A_0-A_1) / A_0 \times 100)$, Where A_0 was the absorbance of the control (blank, without *Anisochilus carnosus* extract) and A_1 was the absorbance in the presence of the *Anisochilus carnosus* extract.

Hydrogen peroxide scavenging activity assay:

Hydrogen peroxide scavenging activity of the *Anisochilus carnosus* extract was estimated by replacement titration [9]. Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 2.0 ml of various concentrations of *Anisochilus carnosus* extract were mixed, followed by 2 drops of 3 % ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 SMKI. The resulting solution was titrated with 5.09 mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as: Inhibition = $(V_0 - V_1) / V_0 \times 100$, Where V_0 was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without *Anisochilus carnosus* extract), V_1 was the volume of NaS_2O_3 solution used in the presence of the *Anisochilus carnosus* extract.

Animal model

Swiss albino mice of either sex (30 animals) were divided in to five groups comprising six mice in each group. Group I (control) received only distilled water. Group II (positive control) received 250 mg/kg bw paracetamol suspension only. Group III received paracetamol suspension 250 mg/ kg body weight (bw) + *Anisochilus carnosus* whole plant extracts (ACWPE) 200 mg/kg bw of animals. Group IV received paracetamol suspension 250 mg/kg bw + ACWPE 400 mg/kg. Group V received paracetamol suspension 250 mg/kg + silymarin 100 mg/kg. The ACWPE was administered 2 hour after the administration of paracetamol suspension. All the treatments were given orally by means of a gastric tube.

Collection of blood samples

The treatments were continued for 7 days and on the eighth day all animals were sacrificed under light ether anesthesia and blood collected without the use of anti-coagulant for serum preparation. The blood samples were collected by direct cardiac puncture and allowed to stand for 10 min before being centrifuged at 2,000 rpm for 10 min and the serum was collected using rubber micropipette. The levels of alkaline phosphatase (ALP) was analysed by the method of Wright et al.[10], alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT) were analysed according to Reitman and Frankel [11].

Histopathological studies

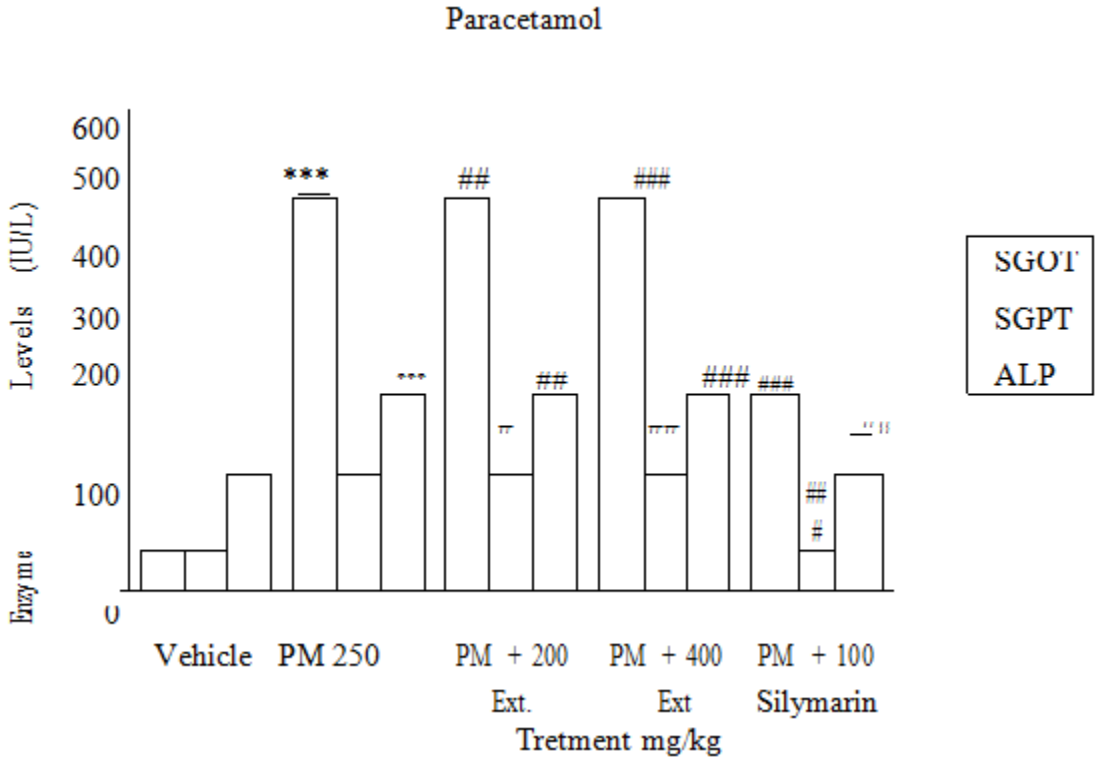
The liver tissue was fixed in 10 % formalin and 5µ sections were cut, stained with haematoxylin and eosin and observed under light microscope (Figure 1-5).

Statistical analysis

Data for the *in vitro* antioxidant activity was expressed as Mean ± SD from three separate observations. Data for hepatoprotective activities were expressed as Mean ± SEM from 6 mice in each group. Hepatoprotective activity were analysed statistically using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The minimum level of significance was fixed at P<0.05.

RESULTS AND DISCUSSION

The *in vitro* antioxidant activities of *A. carnosus* exhibited concentration dependent free radical scavenging activity when compared with ascorbic acid as a reference standard. The above effect may be due to the presence of terpeoids (α-amyrin and β-amyrin) and flavonoids as suggested by Francisco *et al* [12] and Sen *et al* [13].



Graph 1: Effect of ACWPE on SGOT, SGPT and ALP of paracetamol induced hepatotoxicity in mice

Values are in Mean ±SEM (n=6) ANOVA followed by Tukey's Test.

****P<0.001 Vs Control group # P<0.05, ## P<0.01, ### P<0.001 Vs Paracetamol group*

Figures 1 to 5 are histological sections of liver stained with haematoxlin and eosin. X 400

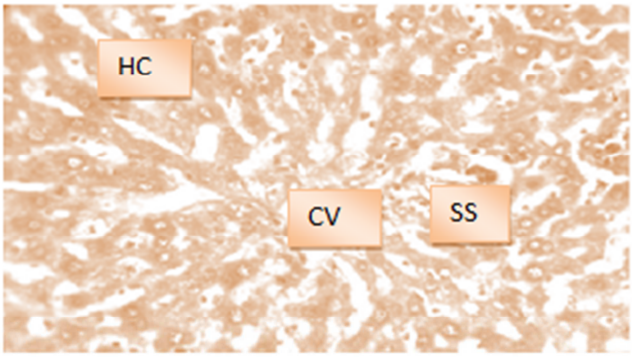


Figure 1

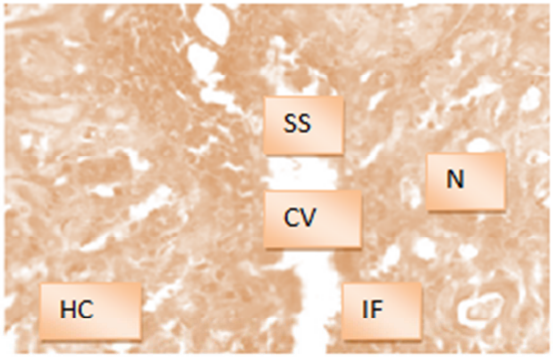


Figure 2

Fig. 1: Mice liver in control group showing normal hepatic cells (HC) with central vein (CV) and sinusoidal dilation (SS)

Fig.2: Mice liver treated with paracetamol exhibited severe necrosis (N) with disappearance of hepatocytes, areas of inflammation (IF) and increased sinusoidal spaces (SS)

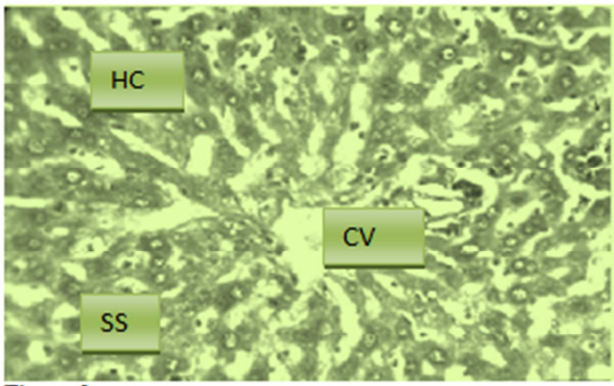


Figure 3

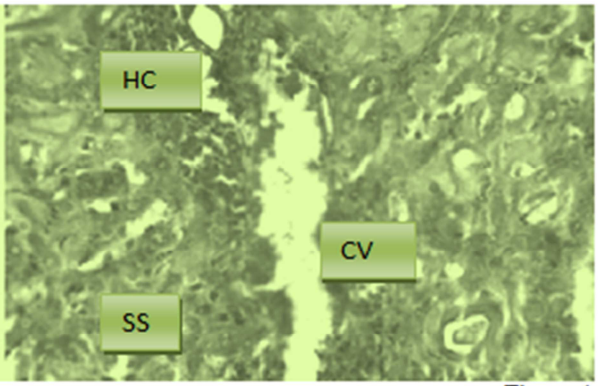


Figure 4

Fig. 3: Mice liver treated with 200 mg/kg ACWPE and paracetamol exhibited mild degree of necrosis (N), normalization of cells (HC) with central vein (CV) and reduced sinusoidal dilation (SS)

Fig. 4: Mice liver treated with 400 mg/kg ACWPE and paracetamol showed normalization of hepatocytes (HC) with some regenerating hepatic cells, reduced sinusoidal dilation (SS) along with mild inflammogens (I)

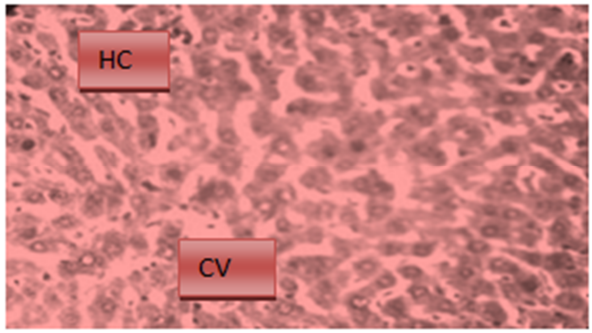


Fig. 5: Mice liver treated with silymarin and paracetamol exhibited normal hepatocytes (HC) with central vein (CV)

Table 1: Effect of *A. carnosus* on serum marker enzymes in paracetamol induced hepatotoxicity in mice

Treatment	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	-	43.2 ± 2.462	24.6 ± 3.238	89.72 ± 4.52
Paracetamol	250	634.73 ± 3.542***	105 ± 4.641***	232 ± 4.043***
PM + 200 mg/kg ACWPE	200	495 ± 3.153 ##	105.17 ± 1.376 #	187 ± 4.018 ##
PM + 400 mg/kg ACWPE	400	457 ± 5.756 ###	97.32 ± 1.536 ##	179.15 ± 5.679 ###
PM +100 mg/kg silymarin	100	173.15 ±2.123 ###	44.89 ± 3.25 ###	125.34 ± 3.363 ###

*Values are Mean ±SEM, n = 6, *** (P<0.001), # (P<0.05), ## (P<0.01). Paracetamol Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. SGPT, SGOT and ALP (aspartate aminotranseferase, alanine aminotranseferase and alkaline phosphatase)*

The effects of *A. carnosus* on different serum marker enzymes are represented in Table 1. The levels of alkaline phosphatase (ALP), alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT) were markedly elevated in paracetamol treated group as compared to control indicating hepatic damage. The ACWPE at dose of 200 and 400 mg/kg of bw exhibited significant decrease in SGOT, SGPT and ALP levels as compared with paracetamol group. The effect of ACWPE was comparable with that of standard drug silymarin (Table 1). Histopathological studies of mice liver tissue (Group I) show normal hepatic cells (HC) with central vein (CV) and sinusoidal dilation (SS) (Fig. 1). In paracetamol treated group (Group II) hepatotoxicity was observed by severe necrosis (N), disappearance of hepatocytes in areas of inflammation (IF) and increased sinusoidal spaces (Fig. 2). Mild degree of necrosis (N) with normalization of cells (HC), central vein (CV) and reduced sinusoidal dilation was observed in Group III (Fig.3), whereas the Group IV animals showed normalization of hepatocytes (HC) with some regenerating hepatic cells, reduced sinusoidal dilation (SS) along with mild inflammogens (Fig. 4). The Group V animals treated with standard drug silymarin showed the normal hepatocytes (HC) with central vein (Fig. 5), hence the present investigation supports the fact that pretreatment of ACWPE at 200 and 400 mg/kg bw can significantly prevent the paracetamol induced hepatotoxicity.

Acetaminophen (paracetamol) is a widely used antipyretic-analgesic drug and produces acute hepatic damage on accidental over dosage. It is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite, N–acetyl–p–benzoquinamine (NAPQI) [14] which is normally conjugated with glutathione and excreted in urine. Overdose of acetaminophen depletes glutathione stores, leading to

accumulation of NAPQI, mitochondrial dysfunction [15] and the development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in acetaminophen bioactivation to NAPQI. The P450 2E1 have been suggested to be primary enzymes for acetaminophen bio-activation in liver microsomes [16]. Studies demonstrated that acetaminophen induced hepatotoxicity can be modulated by substances that influence P450 activity [17]. In the assessment of liver damage by acetaminophen the determination of enzyme levels such as SGOT, SGPT is largely used. Necrosis or membrane damage releases the enzyme into systemic circulation and hence it can be measured in the serum. High levels of SGOT indicate liver damage that is caused by viral hepatitis as well as cardiac infarction and muscle injury, SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, SGPT is more specific to the liver and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [18].

Increase in serum level of ALP is suggested to be due to increased synthesis, in presence of increasing biliary pressure [19]. Administration of acetaminophen caused a significant ($P<0.001$)

Elevation in the level of enzymes studied, when compared to control. Study showed a significant decrease of these enzymes on pretreatment of the *A. carnosus* whole plant extract at a dose of 200 and 400 mg/kg bw (Table 1; Graph 1).

The reversal of increased serum enzymes in acetaminophen- induced liver damage, by whole plant extract of *A. carnosus* may be due to its membrane stabilizing activity. Thus acting as stabilizing agent, whole plant extract increased the stability of membrane and simultaneously prevented the intracellular leakage of enzymes. This is agreement with the commonly accepted that the serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [20].

Effective control of ALP levels points towards an early improvement in the secretary mechanism of the hepatic cells. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin. The plant extract decreased acetaminophen induced elevated enzyme levels in test group, indicating the protection of structural integrity of hepatic cell membrane of damaged liver cells. Extensive vascular degenerative changes and centrilobular necrosis in hepatocytes was produced by acetaminophen. The pretreatment with ethanolic extract of *A. carnosus* produced only mild degenerative changes and absence of centrilobular necrosis, indicating its hepatoprotective efficiency. Preliminary photochemical studies revealed the presence of flavonoids and terpenoids in ACWPE. The terpenoids and flavonoids are well known for their hepatoprotective potential [21-25]. The observed hepatoprotective activity of *A. carnosus* may be due to the presence of flavonoids and terpenoids.

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